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PERSPECTIVES IN BASIC SCIENCE

Multiple facets of macrophages in renal injury

DAVID C. KLUTH,[1] LARS-PETER ERWIG, and ANDREW J. REES

MRC Centre for Inflammation Research, University of Edinburgh, Edinburgh, Scotland, United Kingdom; Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, Scotland, United Kingdom

Multiple facets of macrophages in renal injury. Macrophage infiltration is a common feature of renal disease and their presence has been synonymous with tissue damage and progressive renal failure. More recently work has focused on the heterogeneity of macrophage activation and in particular their ability to curtail inflammation and restore normal function. This has led to the view that it is macrophage function rather than their number that is important in determining the outcome of inflammatory disease. This review will focus on the pathways that regulate macrophage infiltration and activation and how these could be manipulated to control renal inflammatory disease. In particular, the ability of specific cell surface receptors and intracellular signaling pathways to control macrophage activation and how macrophages can be genetically manipulated to develop properties that favor resolution over ongoing injury.

Macrophage infiltration is one of the most striking features of glomerular and tubulointerstitial disease, and the degree of mononuclear cell infiltrate has been used to predict subsequent progression [1]. Nephrologists have generally viewed macrophages as cells that, on entry to the kidney, were activated to produce a range of cytokines, chemokines, and other mediators that were responsible for the tissue injury and subsequent scarring. This was part of a more general paradigm that emphasized the importance of the destructive properties of inflammation in host defense against infection and for tumor destruction, and consequently the particular macrophage properties able to prosecute it.

This restricted view has changed radically over the last 5 years. Inflammation is now viewed more globally as a highly regulated response to injury designed to restore normal function with the minimal tissue damage possible. This highlights a much more complex role for macrophages in maintaining tissue integrity that includes patrolling tissues to sense the presence of infection and other types of injury; responding appropriately and often destructively to the damage identified; promoting resolution of acute inflammation; and then facilitating tissue repair and restoration of normal function. This raises the possibility of harnessing the macrophage's natural reparative properties therapeutically to curtail immune-mediated injury.

The challenge is how to apply this approach to patients with progressive renal injury. The purpose of this review is to summarize knowledge about macrophage function and the activation states responsible for it. The emphasis will be on in vivo studies, especially those of renal inflammation and the insights they provide into what determines the outcome of inflammation. We will discuss factors that control macrophage entry into inflamed renal tissue and show that macrophage function rather than number usually dictates the severity of injury. In addition we will discuss how therapeutic interventions alter macrophage phenotype and how macrophages can be genetically modified to ameliorate renal inflammation.

MACROPHAGES IN RENAL DISEASE

In many renal diseases large numbers of infiltrating macrophages can be identified, in particular in antineutrophil cytoplasmic antibodies (ANCA)-positive glomerulonephritis, lupus nephritis, cryoglobulinemia, mesangioproliferative glomerulonephritis, and IgA nephropathy [2], and interstitial macrophages are a common feature of most forms of progressive renal damage including those not generally considered to be inflammatory such as diabetic nephropathy. Similarly macrophage infiltration is a feature of a wide range of experimental models of glomerulonephritis, including nephrotoxic nephritis, Thy 1 nephritis, murine models of lupus nephritis, Heymann's nephritis, and immune complex glomerulonephritis [3–6], as well as in a number of nonimmune models of progressive renal damage such as renal ablation, puromycin, and adriamycin nephrosis and unilateral ureteric obstruction [7].

Initial studies focused on the pathogenic properties of macrophages by using different approaches to reduce

[1]D.C.K. is an NKRF Senior Research Fellow.

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macrophage numbers. In a model of nephrotoxic nephritis in rabbits treatment with an antimacrophage antibody reduced the severity of inflammation, while induction of leukopenia by nitrogen mustard protected from development of disease which could be reconstituted by injection of peritoneal macrophages [8, 9]. An updated version of these experiments confirmed the pathogenic potential of macrophages in nephrotoxic nephritis. Leukopenia was induced by cyclophosphamide and disease could be reconstituted by injection either bone marrow–derived macrophages (BMDM) or a macrophage cell line NR8383. The severity of injury seen correlated with the numbers of macrophages injected [10]. Similarly stimulation of macrophages with interferon-γ (IFN-γ) before injection increased the severity of proteinuria, showing that the state of macrophage activation is critical in determining outcome [11]. In anti-Thy 1.1 glomerulonephritis, depletion of macrophages using clodronate liposomes led to a reduction in mesangial matrix expansion without altering proteinuria and haematuria [12]. In puromycin nephrosis and rat renal ablation model whole body radiation prevented progressive glomerular injury with a reduction in glomerular and interstitial macrophage infiltration [13, 14].

MACROPHAGE FUNCTION IN INFLAMED GLOMERULI

The critical question is what functions do macrophages perform in inflamed glomeruli and what determines this? Macrophages are capable of producing a wide range of potentially cytotoxic products, including proteolytic enzymes, reactive oxygen and nitrogen species, eicosanoids, proinflammatory cytokines, and chemokines. Studies over many years demonstrate production of these mediators by renal macrophages. Thus macrophages isolated from nephritic glomeruli in rats and rabbits generate reactive oxygen species [15, 16]. Similarly nephritic glomeruli from rats produce large amounts of nitric oxide [17] and in both nephrotoxic nephritis and Heymann's nephritis macrophages are the principal source [18]. The exact contribution of nitric oxide to glomerular injury remains controversial with an early study showing that decreasing nitric oxide production by L-arginine depletion in rat nephrotoxic nephritis exacerbated proteinuria [19], while a more recent study using synthetic inducible nitric oxide synthase (iNOS) inhibitor in Wistar-Kyoto (WKY) rat nephrotoxic nephritis reduced crescent formation [20]. In vitro macrophage-derived nitric oxide and tumor necrosis factor-α (TNF-α) induce mesangial cell apoptosis [21], and uptake of apoptotic mesangial cells down-regulates macrophage inflammatory mediators [22]. These studies demonstrate that macrophages within acutely injured glomeruli have characteristics typical of histotoxic cells.

Production of proinflammatory cytokines by macrophages may also play a key role. TNF-α is produced by glomerular macrophages in both MRL/lpr lupus mice and rabbits with nephrotoxic nephritis [23–25]. Similarly interleukin (IL)-1β production has been shown [26] and this has led to a number of studies to inhibit these cytokines. Blockade of either TNF-α or IL-1β was able to suppress glomerular inflammation in a number of disease models [27]. Treatment of rats with nephrotoxic nephritis with IL-1 receptor antagonist (IL-1ra) reduced macrophage infiltration, histologic markers of injury, and the level of proteinuria [28, 29] and IL-1ra was able to have this effect even when therapy was commenced 7 days after onset of disease [30]. In crescentic glomerulonephritis in WKY rats soluble TNF receptor p55, which acts as a TNF antagonist, reduced the severity of nephritis and crescent formation when administered before or after the onset of disease [31]. Anti-TNF therapy using humanized mouse monoclonal antibodies is now used in the treatment of a number of autoimmune diseases including systemic vasculitis [32]. Both IL-1β and TNF-α signal through nuclear factor-κB (NF-κB) and not surprisingly inhibiting NF-κB signaling cascade with pyrrolidine dithiocarbamate (PDTC) (a relatively nonspecific inhibitor) reduced inflammation and glomerular expression of IL-1β, monocyte chemoattractant protein-1 (MCP-1), and iNOS [33]. Macrophage migration inhibitory factor (MIF) is another inflammatory cytokine with an important role. It was originally identified as a T cell and macrophage-derived cytokine involved in delayed type hypersensitivity reactions and in countering the actions of steroids [34]. It is also produced by endothelium and tubular cells and its expression is up-regulated in nephrotoxic nephritis in rats [35] and in human glomerulonephritides [36]. Passive immunisation against MIF reduced macrophage infiltration and severity of glomerular inflammation even in established disease [37, 38].

Studies inhibiting proinflammatory mediators have identified some of the important inflammatory mediators responsible for renal injury. However systemic administration of agonists/antagonists potentially affects all cells involved in renal inflammation rather than specifically macrophages. Despite these limitations macrophage activation on infiltration into inflamed renal tissue has been convincingly shown to cause injury (Fig. 1). This in turn has directed research to determine different ways in which macrophages can be activated and how these could be manipulated.

MACROPHAGE ACTIVATION

Classical activation

Macrophages evolved to deal with microbial infection and a range of receptors on the cell surface recognize

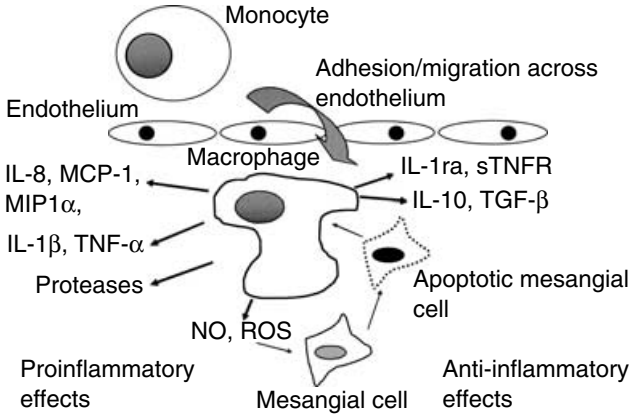

Fig. 1. Macrophage function on entry into inflamed tissue. Monocytes adhere to endothelium activated by the inflammatory process via integrins and chemoadhesins following a chemotactic gradient [121]. Once within the inflamed tissue they are capable of both promoting inflammation by release of chemokines, proinflammatory cytokines, proteolytic enzymes, and reactive oxygen and nitrogen species and of promoting resolution by release of cytokine inhibitors, regulatory cytokines [interleukin (IL)-10 or transforming growth facto-β (TGF-β)] and removing apoptotic cells with consequent anti-inflammatory effect [22]. Abbreviations are: MCP-1, monocyte chemoattractant protein-1; IL-1ra, IL-1 receptor antagonist; TNFR, tumor necrosis factor receptor; NO, nitric oxide; ROS, reactive oxygen species; MIP-1α, macrophage inflammatory protein-1α.

pathogen-associated molecular patterns (PAMPs), which include Toll-like receptors (TLRs), mannose receptor and scavenger receptor family (Table 1). TLRs recognize a range of microbial products including lipopolysaccharide (LPS) acting on TLR4, bacterial unmethylated CpG DNA acting via TLR9 and viral double-stranded (ds)RNA acting via TLR3 [39]. These in turn lead to activation of intracellular signaling pathways, including NF-κB and the mitogen-activated protein kinase (MAPK). Classical activation involves stimulation of macrophages with two signals. The first is the engagement of TLR and the second is provided by IFN-γ from T-helper lymphocytes or natural killer cells. This leads to macrophage production of nitric oxide and reactive oxygen species, production of other proinflammatory cytokines, including TNF-α and IL-12 and expression of major histocompatibility complex (MHC) class II and costimulatory molecules which promote antigen presentation. This response is designed to enhance microbial killing and activate adaptive immunity [40]. There is increasing evidence that as well as binding to microbial products TLRs also recognize endogenous ligands released by damaged tissues such as heat-shock protein 60 and 70 which are ligands for TLR4 [41] and dsDNA which interacts with TLR9 [42]. Of note administration of unmethylated CpG exacerbated disease severity in immune complex glomerulonephritis by a TLR-dependent mechanism [43]. Classically activated macrophages have been found at sites of immune-mediated inflammation, including glomerulonephritis [44], especially during the initial induction of the inflammatory response. However, it is important to recognize macrophages can be activated in other ways and display much greater functional heterogeneity than previously envisioned [45].

Alternative activation

The first example of this was the characterization of the alternatively activated macrophage which develop after exposure to IL-4 or IL-13 [46, 47]. These cells show increased expression of mannose receptor and MHC class II, increased endocytosis, decreased nitric oxide production due to both decreased expression of iNOS and increased production of arginase and increased expression of IL-1 decoy receptor and IL-1ra [48]. Thus these cells have decreased killing of intracellular organisms but increased matrix production suggesting a role in tissue repair [49].

Type II–activated macrophage

More recently Mosser [50] has identified a "type II–activated macrophage." They found that exposure of macrophages to activating stimuli such as LPS or CD40L in the presence of IgG immune complexes resulted in increased IL-10 expression and reduced IL-12 expression but with preservation of other proinflammatory cytokines such as TNF-α and IL-6 [51, 52]. These cells in vivo favor the development of Th2 type immune responses with increased T-cell IL-4 production and IgG class switching by B cells [53].

Macrophage uptake of apoptotic cells

Removal of dying cells by macrophages is an essential component of normal development and in the resolution of inflammation allowing uptake of dying cells before they release their potentially toxic contents [54]. Uptake of apoptotic cells results in development of macrophage with immune inhibitory properties. Phagocyte ingestion of apoptotic cells is a complex process involving a range of cell surface receptors both on the apoptotic cells and macrophages as well as various bridging molecules between them [54]. Macrophages which have ingested apoptotic cells develop anti-inflammatory properties with increased expression of transforming growth factor-β (TGF-β) and prostaglandin E_2 (PGE_2) and decreased production of IL-8, TNF-α, and IL-1β [55] and on further stimulation with LPS macrophages activated in this way increase IL-10 production while decreasing IL-12 [56]. In addition, macrophages that have taken up apoptotic cells are also able to suppress T-cell proliferative responses whereas uptake of necrotic cells has the opposite effect [57]. Defective uptake of apoptotic cells, for example in mice deficient in the C1q complement

Table 1. Pathogen associated molecular pattern recognition molecules on macrophage cell surface

Receptor	Pathogen molecular pattern
TLR2 plus 1 or 6 (forming cell surface receptor heterodimers) [41]	Zymosan, lipoproteins from *Mycobacterium tuberculosis*, *Borrelia burgdorfei*, *Treponema pallidum*, and *Mycoplasma fermentans*, mycobacterial lipoarabinomannan, peptidoglycan, *Treponema* glycolipid, *Trypanosoma cruzi* glycophosphatidylinositol anchor
TLR3 [39]	Double-stranded RNA
TLR4 [41]	Lipopolysaccharide (gram-negative bacteria), lipotechoic acid (gram-positive bacteria, *Mycobacterium tuberculosis*, fusion proteins on respiratory syncytial virus (RSV), heat shock protein 60 (HSP60) on *Chlamydia pneumoniae*
TLR5 [41]	Flagellin
TLR9 [41]	Unmethylated CpG DNA
Mannose receptor [196]	Oligosaccharides, involved in recognition of bacteria (*Klebsiella pnemoniae*, *Mycobacterium tuberculosis*) fungi (*Crytococcus neoformans*) viruses [human immunodeficiency virus (HIV)] and protozoa (*Pneumocystis carinii*)
Scavenger receptor A [197]	Lipotechoic acid, lipopolysaccharide, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria meningitides, Clostridium perfringens*
MARCO (macrophage receptor with a collagenous structure) [198]	*Escherichia coli*, *Staphylococcus aureus*
Dectin-1 [199]	β-glucans on zymosan, involved in fungal recognition.

component, leads to generation of autoantibodies and in a proportion of mice the spontaneous development of glomerulonephritis with increased local apoptotic bodies [58]. Similarly, mice deficient in CD44 (hyalauronan receptor) develop more severe bleomycin induced lung inflammation due in part to impaired clearance of apoptotic neutrophils [59].

As these studies show a large number of receptors can potentially be involved in apoptotic cell uptake but the recently identified phosphatidylserine receptor (PSR) plays a significant role in development of the anti-inflammatory phenotype [55]. Utilization of this mechanism has not been directly applied to renal disease, however, in a model of lung inflammation induced by LPS the intratracheal instillation of apoptotic neutrophils markedly reduced local chemokine production and total inflammatory cell infiltrate and this was dependent on macrophage uptake of the apoptotic cells and subsequent TGF-β production [60]. It is likely that other apoptotic cell receptors will play a similar role.

As yet no direct approaches to manipulate the uptake of apoptotic cells have been tried in renal inflammation though some promising agents exist. These include lipoxins which are endogenously produced anti-inflammatory eicosanoids, for which synthetic analogues also exist. Lipoxins increase macrophage uptake of apoptotic neutrophils and decrease mesangial cell proliferation [61] and studies are currently assessing their ability to influence renal inflammation [62, 63]. The augmented uptake induced by lipoxins was not inhibited by blockade of PSR showing the involvement of other macrophage receptors in apoptotic cell removal.

These examples have highlighted that as well as being involved in tissue damage macrophages can be intimately involved in regulation and repair (Table 2). We will now focus on the mechanisms that control these different states of activation.

Table 2. Macrophage activation states

Classically activated [45]	↑ iNOS, IL-1β, TNF-α, IL-6, HMGB1, IL-8, MCP-1, MHC class II expression
Alternatively activated (IL-4/IL-13) [45]	↑ Mannose receptor, MHC class II, arginase, FIZZ1/Ym1, IL-1ra↓ iNOS, TNF-α, IL-6
Type II activated [50]	↑ IL-10, TNF-α, IL-6, MHC class II↓ IL-12
Uptake of apoptotic cell [54]	↑ TGF-β, PGE_2,↓ TNF-α, IL-8, MCP-1
IL-10 activated [189]	↓ IL-1β, TNF-α, IL-12, IL-6, IL-8, iNOS, MHC class II↑ Soluble TNF receptors, IL-1ra
Steroid treated	↑ IL-10, uptake of apoptotic cells↓ IL-12, TNF-α, IL-1β, IL-6

Abbreviations are: IL, interleukin; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-α; HMGB1, high mobility group B1; MCP-1, monocyte chemoattractant protein-1; MHC, major histocompatibility complex; IL-1ra, IL-1 receptor antagonist; TGF-β, transforming growth factor-β; PGE_2, prostaglandin E_2.

CONTROL OF MACROPHAGE FUNCTION VIA SURFACE RECEPTORS

As inflammation can have such deleterious consequences for the host it is a tightly regulated system and a number of receptors on macrophages have been identified which provide checks on the inflammatory cascade. Thus, a key aspect of the inflammatory response is the pairing of activation with inhibitory regulatory signals and the loss of these signals often leads to unchecked immune activation [64]. A number of receptors have a negative regulatory effect on leukocyte function and often inhibit the function of immunoreceptor tyrosine-based activation motif (ITAM) containing receptors. These counter-regulatory signals are provided by the immunoreceptor tyrosine-based inhibitory motif (ITIM). The clustering of these motifs leads to the binding and activation of SHP-1, which is a tyrosine phosphatase, and SHIP, which is an inositol phosphatase which transduce the negative regulatory signal.

Table 3. Functions of suppressors of cytokine signalling (SOCS) in control of cell signaling [87]

Suppresors of cytokine signaling	Induced	Inhibits signaling
Cytokine-induced SRC-homology-2 protein (CIS) [87]	IL-2, IL-3, IL6, IL-10	**IL-2**, IL-3, Epo, IGF-1
SOCS1 [200]	IL-2, IL-4, IL-6, IL-10, IFN-α/β/γ, TNF	**IFN-γ**, **IFN-β**, IL-2, IL-4, IL-6, M-CSF
SOCS2	IL-2, IL-6, growth hormone	**Growth hormone**
SOCS3 [88, 89]	IL-6, IL-10, LPS, IL-11, IL-22, TNF	**IL-6**, IFN-γ, Epo

Abbreviations are: IL, interleukin; INF, interferon; TNF, tumor necrosis factor; Epo, erythropoietin; IGF-1, insulin-like growth factor-1; M-CSF, monocyte-colony stimulating factor.

CIS and SOCS1 to 3 have been those best characterized and the cytokine or growth factor in bold represent those for which a physiologic role in control of signaling is most likely. The role of SOCS4 to 7 in control of macrophage signaling is at this stage is unclear.

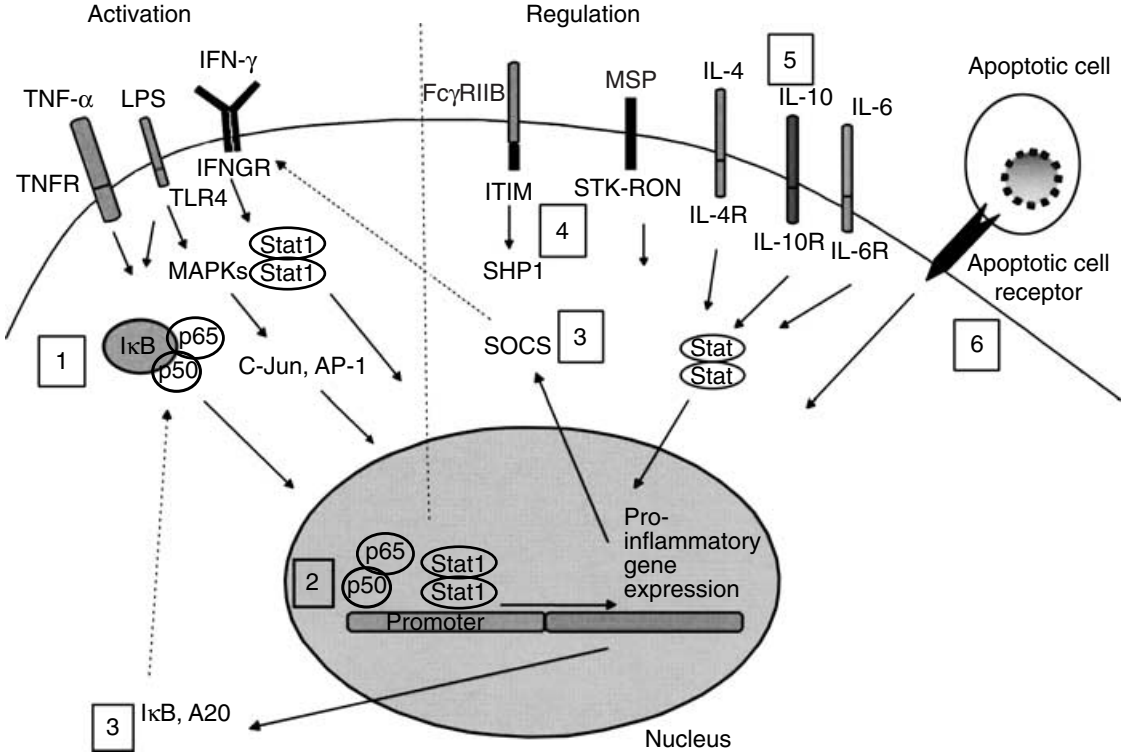

Fig. 2. Regulation of macrophage function. Macrophage function is tightly regulated at a number of levels from the cell surface to the nucleus. (**1**) Proinflammatory cytokines and pathogen associated molecular patterns activate cell surface receptors which leads to activation of a number of intracellular signalling pathways including STAT1, nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs) which in turn leads to the translocation of transcription factors to the nucleus (**2**) and activation a range of genes involved in the pro-inflammatory response [82]. (**3**) Activation also increases production of molecules such as SOCS1 which inhibits activation by the interferon-γ (IFN-γ) receptor and IκB and A20 which inhibit actions of NF-κB [83, 85]. (**4**) In addition, certain receptors such as FcγRIIB contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) which activates SHP1 which is a phosphatase capable of negatively regulating a number of kinase related pathways. (**5**) Other cells surface receptors activate signaling cascades which modulate and inhibit the inflammatory response and lead to a modified state of activation which is principally anti-inflammatory in nature. (**6**) Uptake of apoptotic cells via the phosphatidylserine receptor (PSR) receptor leads to transforming growth factor-β (TGF-β) production and reduced responsiveness to proinflammatory mediators. Abbreviations are: TNFR, TNF receptor; TLR4, Toll-like receptor 4; IFNGR, IFN-γ receptor; SHP1, Src homology 2 domain phosphatase; SOCS, suppressors of cytokine signalling; MSP, macrophage stimulating protein.

A well characterized example of these competing influences is provided by the Fcγ receptor. FcγRIII is an ITAM containing receptor which binds IgG immune complexes and activates macrophages, including promoting phagocytosis and release of pro-inflammatory cytokines. FcγRIIB by contrast is an ITIM receptor which on binding IgG inhibits ITAM-triggered activation. The importance of this pathway is illustrated by the observation that FcγRIIB-deficient mice develop more severe pulmonary inflammation in an immune complex-induced disease with increased production of pro-inflammatory cytokines by macrophages [65]. In addition these mice develop a Goodpasture's syndrome on immunization with type IV collagen and become susceptible to collagen-induced arthritis both marked by macrophage activation [66, 67]. The therapeutic significance of this receptor was shown in a murine model of immune thrombocytopenia where the protective

effect of intravenous immunoglobulin was shown to be dependent on the FcγRIIB receptor, which in turn may explain the value of intravenous immunoglobulin in treating immune-mediated inflammation [68].

Other counterregulatory receptors include CD200 receptor (CD200-R) which is a membrane glycoprotein expressed by myeloid cells [69] and recognizes CD200 (OX-2) expressed on many cell types, including endothelium. In a mouse model of experimental allergic encephalomyelitis (EAE) inhibition of CD200-R using an antibody worsened disease severity. In CD200-deficient mice, EAE onset was accelerated, experimental uveoretinitis was more severe, and mice normally resistant to collagen-induced arthritis developed disease [70, 71]. Thus CD200-macrophage interaction could be viewed as providing an early restraint on macrophage responses at the tissue level.

Another recently identified regulator of macrophage function is the CD47-signal regulatory protein-alpha (SIRPα) (CD172a) interaction [72]. CD47 is ubiquitously expressed, while SIRPα is limited to myeloid cells. Engagement of SIRPα activates ITIM which leads to recruitment and activation of SHP-1 and SHP-2, which in turn inhibit tyrosine kinase-dependent activation pathways [73]. Activation of SIRPα inhibits maturation of dendritic cells with reduction of costimulatory molecule expression and suppresses production of TNF-α, IL-12, and IL-6 in response to LPS [74, 75], while expression of CD47 on red blood cells prevents their phagocytosis by macrophages by activating SIRPα [76].

Macrophage-stimulating protein (MSP) is produced as a promolecule in the liver which is then activated by factors from the coagulation cascade at sites of local inflammation. MSP binds to stem cell–derived tyrosine kinase receptor (STK/RON) which is expressed on macrophages (reviewed in [77]). MSP-RON interaction inhibits LPS and IFN-γ–induced nitric oxide production [78], increases arginase expression, and up-regulates other genes associated with alternative macrophage activation, including scavenger receptor A and IL-1ra [79]. In addition, mice which lack STK/RON have increased inflammation in IFN-γ–mediated delayed-type hypersensitivity response, increased susceptibility to LPS-induced shock, and macrophages isolated from these mice produce elevated levels of nitric oxide in response to IFN-γ [80]. There is also evidence that LPS can reduce RON expression in vitro and in vivo [81]. Thus this system may provide an important check on the control of macrophage-mediated inflammation although its role in models of immune disease has not been assessed.

These examples show clear evidence of negative regulatory pathways controlling macrophage function which provide additional checkpoints in the inflammatory response. Other examples include more classical cytokine receptor responses such IL-4, IL-10, and IL-13 which can combine with the above signals to significantly alter macrophage function.

SIGNALING PATHWAYS CONTROLLING MACROPHAGE FUNCTION

The previous sections illustrates that macrophage function is regulated by the signals impinging on surface receptors and the interplay between opposing surface receptors. Ultimately, events on the cell surface lead to activation of signaling cascades and specifically to activation of transcription factors. Two of the key groups of transcription factors are NF-κB activated by inflammatory mediators such TNF-α, IL-1β, and LPS and STAT family which transduce signals from IFNs, IL-12, IL-6, IL-4, IL-13, and IL-10 [82]. As with cell surface receptors these signals are carefully regulated within the cell to prevent excessive activation. NF-κB is family of 5 proteins (p65, Rel-A, c-Rel, p50, and p52) which are bound by an inhibitory protein IκB in the cytosol [83]. Activation of signalling cascades leads to the phosphorylation of IκB by IκB kinases, which results in the ubiquitination of IκB, degradation by the 26S proteosome and release of NF-κB molecules. These in turn form heterodimers and homodimers which enter the nucleus and activate transcription of NF-κB–dependent genes which include TNF-α, IL-1β, IL-8, leukocyte function-associated molecule-1 (LFA-1), very late antigen-4 (VLA-4), costimulatory molecules CD80 and CD86, and IL-10. NF-κB also increases expression of IκB which then binds and removes NF-κB from the nucleus [84]. Similarly A20 is a cytoplasmic protein which can also inhibit NF-κB activation [85] and its expression is increased by TNF-α, and may have particular relevance in terminating TNF-induced inflammation [86].

The JAK-STAT family of proteins are responsible for signaling from a wide range of cytokines in macrophages including IFN-γ (STAT1), IL-6 (STAT1 and 3), IL-10 (STAT3), and IL-12 (STAT4) [82]. IFN-γ and IL-12 both promote the inflammatory response while IL-10 provides critical regulation of inflammation and as later sections will show is of potential therapeutic value. Negative regulation of these signaling pathways is provided by members of the suppressor of cytokine signaling (SOCS) family of which eight have been identified so far (Table 2) [87]. The best characterized include SOCS-1 which is up-regulated by IFN-γ and in turn binds to and inhibits kinase activity of JAK2 to inhibit IFN-γ signaling, while SOCS3 has recently been identified as the key inhibitor of the IL-6-STAT3 pathway [88, 89]. Again these pathways prevent unrestrained inflammatory activity which is seen in the $SOCS1^{-/-}$ mouse [90].

Other transcription factors involved in the regulation of macrophage activation include peroxisome proliferators-activated receptor-γ (PPAR-γ) [91]. This family of transcription factors was originally identified

for their role in glucose and lipid metabolism; however, they are also involved in control of inflammation. PPAR-γ expression is increased in macrophages by IL-4 [92] and PPAR-γ ligands, including the thiazolidinedione class of type II diabetic drugs, result in reduced IL-6, TNF-α, IL-1β and IL-12 expression [93] and decreased iNOS expression [94]. Thus cytokines cell surface proteins and intracellular signaling pathways form a multilayered network in macrophages and other cells involved in the immune response to contain and curtail inflammation.

MODIFICATIONS OF MACROPHAGE FUNCTION BY DISEASE STATES

The importance of the state of macrophages activation in controlling inflammation is most clearly highlighted in diseases which bias macrophage activity to prevent development of the immune response, in particular certain infectious diseases and malignancy [95–97]. Numerous pathogens have developed strategies to subvert macrophage function to dampen the innate inflammatory response and these provide an insight into how macrophages might be manipulated therapeutically. *Yersinia enterocolitica* inhibits the activation of NF-κB in macrophages leading to reduced TNF-α secretion and cells becoming apoptotic with consequent immunosuppressive effects on uptake [98], while *Yersinia pestis* produces a virulence protein, LcrV, which interacts with TLR2 and increases IL-10 production which in turn reduces the inflammatory response [99]. The African swine fever virus which replicates in macrophages produces an IκB-like protein which reduces NF-κB activation [100]. *Leishmania* spp bind to the PSR receptor thus appear to the macrophage as an apoptotic cell with consequent anti-inflammatory release of TGF-β and IL-10 [101, 102]. Similarly uptake of apoptotic T cells by macrophages infected with *Trypanosoma cruzi* dampens their inflammatory response and promotes parasite growth [103]. *Toxoplasma gondii* is another intracellular parasite which on macrophage interaction causes NF-κB and STAT1 activation but prevents their nuclear entry to switch off the antimicrobial response [104].

Tumor-associated macrophages (TAM) are found in nearly all tumors and although they have the potential to kill malignant cells their function is frequently subverted to promote tumor development [96]. Tumors can produce a number of anti-inflammatory cytokines, including IL-4, IL-10, TGF-β, and PGE_2 which suppress cell-mediated immune responses. TAM have a reduced ability to produce IL-12 which is a result of autocrine IL-10 production [105] and similarly dendritic cells from tumors have an immature phenotype and are refractory to stimulation with LPS and IFN-γ due to IL-10 production [106]. In addition, macrophage infiltration actually favors tumor growth and metastasis by production of proteases which degrade extracellular matrix allowing tumor infiltration and angiogenic factors such as vascular endothelial growth factor (VEGF) which promote vascular growth [107]. Thus, the complex relationship between tumor and macrophage shows how deviating macrophage function can prevent an inflammatory response. These examples provide a clear rationale to alter macrophage activation to control local inflammation.

MACROPHAGE LOCALIZATION IN RENAL INFLAMMATION

Macrophage adhesion

The first stage of macrophage involvement in inflammatory renal disease involves their adhesion to and migration across endothelium. The classic paradigm of leukocyte adherence to inflamed endothelium involves lectin interaction which allows leukocyte rolling, this in turn leads to adherence of integrins such as LFA-1 and mac-1 to intercellular adhesion molecule (ICAM-1) and VLA-4 to vascular cell adhesion molecule (VCAM), which firmly binds the leukocyte to the endothelium and allows diapedesis to occur [108]. It is though increasingly clear that different vascular beds within the body and different forms of inflammation may utilize distinct adherence pathways [109]. The role of selectins in glomerular inflammation is still controversial but administration of fucoidan F7 which inhibits L- and P-selectin function failed to alter glomerular leukocyte localisation in nephrotoxic nephritis [110]. In the same disease model P-selectin–deficient mice developed more severe disease most likely due to loss of anti-inflammatory effect of soluble P-selectin [111]. In WKY rats with crescentic glomerulonephritis, by contrast, anti-P-selectin antibody reduced macrophage infiltration and crescent formation [112]; however, this may represent a nuance of the WKY rat model.

Blockade of ICAM-1 or its counter-receptor LFA-1 with antibodies reduced macrophage infiltration in WKY rats with nephrotoxic nephritis [113, 114]; however, these rats have an abnormal immune response and develop very severe glomerular inflammation unlike other rat strains, thus the responses may not be representative. Interestingly administration of anti-CD18 antibodies (counterreceptor to ICAM-1 and 2) in Lewis rats with nephrotoxic nephritis did not reduce macrophage infiltration [115] and similarly blockade of either VCAM or its ligand VLA-4 did not reduce macrophage recruitment in the same disease model [116]. In both of these latter studies macrophage infiltration was not altered however disease was less severe, again demonstrating the disassociation of macrophage numbers from injury. Recently interest has focused on chemoadhesins, including fractalkine and GRO-α (CXCL1). Fractalkine is a chemokine which contains a transmembrane domain

allowing it to be expressed as an endothelial cell surface protein [117], and inhibition of its receptor, CX3CR1, markedly reduced macrophage infiltration in a model of crescentic glomerulonephritis [118]. GRO-α can be immobilized on cell surface proteoglycans which are up-regulated on inflamed endothelium and mediate adherence under conditions of flow [119]. In rats with nephrotoxic nephritis direct blockade of GRO-α receptor on macrophage surface reduced their localization to inflamed glomeruli [120].

ROLE OF CHEMOKINES IN MACROPHAGE LOCALIZATION

Following adhesion to activated endothelium macrophages transmigrate to the focus of injury in response to a chemotactic gradient. A wide range of studies have confirmed their importance in macrophage infiltration in a variety of renal diseases (reviewed in [121]). The main challenge now is to establish which chemokines are critical for pathogenesis, which could potentially favor entry of reparative macrophages and how adhesion and transmigration alter macrophage function. Renal injury, whether toxic, ischemic, or immunologic, can lead to chemokine production by endothelium, mesangial cells, or tubular cells. Many studies have been performed on human biopsies to demonstrate expression of chemokines and their relevant receptors. The most extensively assessed have been MCP-1 and its receptor CCR2, and RANTES, macrophage inflammatory protein-1α and 1β (MIP-1α and MIP-1β) and their receptors CCR1, 3, and 5 due to their well characterized effect on macrophage chemotaxis [122]. MCP-1 and CCR2 have been found in IgA disease [123], crescentic glomerulonephritis, including ANCA-positive disease and lupus nephritis [124–126], and in acute interstitial nephritis [123]. In IgA disease MCP-1 expression was associated with more severe disease and a number of studies have correlated urinary MCP-1 levels with severity of renal injury [127–130]. Similarly MIP-1α, MIP-1β, and RANTES or their receptors have been identified in lupus nephritis, crescentic glomerulonephritis, and IgA disease [126, 129, 131].

The presence of the relevant chemokines as a snap shot in human biopsies tells us relatively little about their functions, in particular how chemokine and chemokine receptor expression varies with stage of disease, in specific inflammatory cells and in different locations within the kidney. For this we have to turn to animal models. Interesting data have been provided by knockout mice for chemokines and their receptors. In mouse nephrotoxic nephritis, MCP-1 was mainly expressed in tubular cells and MCP-1–deficient mice developed less severe tubular injury while glomerular macrophage infiltration and subsequent inflammation were unaltered suggesting that MCP-1 primarily directs macrophages to the interstitium [132]. In the MRL-Fas (lpr) lupus model, MCP-1 deficiency reduced tubular and glomerular macrophage infiltration and protected from severity of disease [133]. In contrast to these results, knockout of the MCP-1 receptor CCR2 led to more severe disease in mice with nephrotoxic nephritis, despite resulting in reduced glomerular macrophage infiltration [134]. Similar to this in the same model knockout of CCR1 (receptor for RANTES and MIP-1α) led to increased renal macrophage infiltration, more severe disease and biased disease toward a Th1 phenotype [135]. These experiments highlight the complexity of chemokine control of leukocyte function as there effects are not limited to the kidney but alter both the biology of cells and their trafficking in other sites within the body that may have important pathologic consequences.

A number of recent studies have focused on the temporal organization of chemokine expression in renal inflammatory models and how these might coordinate macrophage entry. Reversible immune complex glomerulonephritis in mice can be induced by injection of horse apoferritin and in this model MCP-1 and RANTES and their relevant counterreceptors are expressed early on in disease and precede both the peak of biochemical and histologic injury [136] as inflammation resolves chemokines and receptor expression returns to baseline levels. Similar results were observed in a progressive model of inflammation in lupus mice with early expression of MCP-1 and RANTES in glomeruli and interstitium which preceded the influx of mononuclear cells and proinflammatory cytokine expression [137]. In rat nephrotoxic nephritis MCP-1 and RANTES are expressed in glomeruli as inflammation is initiated, but with only limited tubular MCP-1 expression. By contrast tubular levels of osteopontin, a potent macrophage chemokine were elevated [138]. These studies show a coordinated chemokine response both over time and in different locations within the kidney which orchestrate macrophage infiltration.

Despite the reservations about the precise role of chemokines in regulating macrophage infiltration, a number of studies have assessed the impact of inhibiting specific chemokines receptors. Anti-MCP-1 antibody reduced macrophage infiltration, crescent formation, and proteinuria in WKY rats and mice with nephrotoxic nephritis [139–141] and in rats with anti-Thy 1.1 nephritis [142]. In mice with nephrotoxic nephritis blockade of MCP-1 or RANTES resulted in a reduction in glomerular macrophage infiltration but only anti-MCP-1 antibodies reduced crescent formation and collagen deposition [143]. Contrasting with these results in WKY rat nephrotoxic nephritis anti-MCP-1 treatment reduced macrophage infiltration early on but not after first week of disease which suggests that MCP-1 may be the initial chemotactic stimuli but that progression of disease may depend on other stimuli [144]. Herpes virus 8 produces

a chemokine analogue vMIP-II which is able to block macrophage chemotactic responses to MCP-1, MIP-1α and β, RANTES and fractalkine. In WKY rats with crescentic glomerulonephritis vMIP-II reduced macrophage infiltration, crescent formation, and proteinuria while preserving renal function [145]. RANTES function can be blocked by two synthetic antagonists amino-oxypentane RANTES (AOP-RANTES) and Met-RANTES. AOP-RANTES blocked the early influx of macrophages during anti-Thy 1.1 nephritis in rats and murine nephrotoxic nephritis [141, 146]. In immune complex glomerulonephritis, however, treatment with AOP-RANTES or Met-RANTES both reduced glomerular macrophage infiltration but actually resulted in more severe inflammation [147], which emphasizes that it is macrophage function not numbers that will be crucial in controlling disease. More recently, an important role has been proposed for macrophage-derived chemokine (MDC), where blockade of its function in WKY rat nephrotoxic nephritis did not alter initial development of glomerular inflammation but reduced macrophage infiltration and crescent formation from days 7 to 14 of disease [148].

Clearly inhibiting macrophage infiltration represents one approach to modify renal inflammatory disease. An overview of all the studies presented here shows that there are clear species and disease model differences in the relative importance of different chemokines but what is still needed is a better understanding of how chemokine selection dictates the function of infiltrating macrophages and how this changes as disease evolves.

MACROPHAGE ACTIVATION DURING RENAL INFLAMMATION

How macrophages interact with inflamed glomeruli at a single cell level has been less extensively assessed. In part this is due to a relative paucity of markers which clearly delineate different states of activation, although this is being addressed by gene chip analysis of macrophage expression under specific conditions [149]. Work from our group has shown that certain activating signals, such as IFN-γ and TNF-α, programmed macrophages to developed distinct sets of properties in vitro, which included unresponsiveness to other types of activation [150]. Furthermore, macrophages infiltrating acutely inflamed glomeruli of rats with nephrotoxic nephritis display programmed behavior. Operationally, they behave as though programmed by IFN-γ [44], and maintain these characteristics despite systemic administration of alternatively activating cytokines such as IL-4 or TGF-β. Interestingly, the IFN-γ–driven program predominates when immune mediated injury occurs in a TGF-β–rich environment such as the eye [151]. Subsequent observation in anti-Thy 1.1 nephritis has shown that (*1*) macrophage localization itself does not induce programming; (*2*) that all macrophages infiltrating an appropriate environment become programmed shortly after localization; and (*3*) macrophages infiltrating glomeruli at the same time can be programmed in different ways [152]. These observations raise question about the factors that induce macrophage activation and programming at early stages of the inflammatory disease and its consequences for the outcome of the inflammatory process. It provides an important mechanistic insight into how macrophage functional development is influenced by the underlying disease process.

Comparison of macrophage infiltration in ANCA-positive vasculitis and cryoglobulinemic vasculitis shows that a similar heterogeneity exists in human glomeruli. In ANCA-positive disease macrophages were localized at sites of active lesions and were MHC class II and TNF-α–positive while in cryoglobulinemia they were diffusely localized in glomeruli and less than 30% of macrophages had these same markers of activation [153].

Thus macrophages both respond to and produce proinflammatory cytokines within foci of renal inflammation and a recent series of studies has attempted to dissect their relative contributions. Using bone marrow chimeras in knockout mice Timoshanko et al [154, 155] have shown that TNF-α and IL-12 production during nephrotoxic nephritis in mice is mainly dependent on intrinsic renal cells rather than infiltrating leucocytes, while IFN-γ production requires contributions from both [156], with macrophage production potentially as significant as the T-cell contribution [157].

MACROPHAGE FATE DURING PROGRESSIVE INFLAMMATION

After the initial influx of macrophages, there is increasing evidence that the subsequent sustained increase in macrophage numbers is a result both of continuing influx and local proliferation [158]. Expression of proliferating cell nuclear antigen (PCNA) and incorporation of bromodeoxyuridine (BrdU), which are markers of proliferation, can be found in around half of glomerular and tubulointerstitial macrophages in rat nephrotoxic nephritis and rat remnant kidney model [159, 160]. Proliferation was seen mostly in ED-1–positive macrophages and not in ED-2 or ED-3–positive cells which tend to be markers of more mature or activated tissue macrophages. Proliferating macrophages have also been identified in human renal biopsies [161]. Possible growth factors which control this include macrophage-colony stimulating factor (M-CSF) which is involved in macrophage differentiation. Blockade of its receptor, *c-fms*, reduces macrophage accumulation in unilateral ureteric obstruction in mice by markedly reducing macrophage proliferation [162].

The fact that there is continuous macrophage entry into a focus of renal inflammation and proliferation of

macrophages emphasises the need to understand the fate of these cells. The two possibilities are death within the glomerulus or interstitium or emigration to regional lymph nodes. As yet there is no evidence of widespread macrophage apoptosis in inflamed kidneys but the rapid removal of apoptotic cells is beyond the resolution of current techniques. However, cell death could still provide an important contribution. Macrophage migration to regional lymph nodes has been demonstrated in rats with nephrotoxic nephritis [163]. In a mouse model of resolving peritonitis fluorescently labeled macrophage could be tracked to draining lymph nodes and emigrated with a half-life of 48 hours [164]. This process has recently been shown to be actively mediated and partially dependent on VLA-4 and VLA-5 [165]. In our own studies where we have injected labeled adenoviral-transduced macrophages into rats with nephrotoxic nephritis these cells disappear from inflamed glomeruli again with a half-life of approximately 48 hours [166, 167]. These data imply that macrophages are continually trafficking through a site of inflammation and provide a feedback of information to the immune response.

Thus the potential maturation of these cells to dendritic cells which can then inform T-cell responses is a critical issue. A model system for this is provided in vitro where macrophages transmigrate across endothelium placed on a collagen gel [168]. Macrophages reverse migrate across the endothelium with a half-life of 48 hours while some macrophages remained within the gel. When the collagen gel was exposed to proinflammatory stimuli such as LPS or IL-1β the reverse migrated macrophages resembled mature dendritic cells expressing MHC class II, costimulatory molecules and the dendritic cell marker CD83, as well as losing expression of CD14 and Fc receptors. Those that remained behind in the gel resembled tissue macrophages [169]. More recent data suggests that a subset of monocytes expressing FcγrIII (CD16) preferentially become dendritic cells in this setting [170]. It has also been shown that TNF-α and IL-1 promote migration of Langerhans-type dendritic cells from skin [171]. Thus it is likely that macrophages/dendritic cells migrate to regional lymph and are a critical link in the inflammatory response.

IMMUNE MODULATION OF RENAL INFLAMMATORY DISEASE

Given the diverse functions of macrophages in renal inflammatory disease and there ability to respond to a wide range of inputs manipulation of their function provides an attractive approach to alter inflammation.

Cytokines

We have already shown how blockade of proinflammatory cytokines such as TNF-α and IL-1β and chemokines can decrease the inflammatory response. The alternative is to use the cytokine network to promote an anti-inflammatory response [27]. IL-4 is produced by Th2 cells and leads to alternative activation of macrophages. Injection of IL-4 into rats with nephrotoxic nephritis reduced the degree of proteinuria, increased glomerular expression of IL-1ra, and limited macrophage activation as shown by reduced glomerular ED-3+ cells [172]. In Heymann's nephritis treatment with IL-4 prevented the development of proteinuria and reduced glomerular macrophage infiltration, although this effect appeared dependent on IL-4 effects on T cells [173]. IL-10 is a critical regulatory cytokine and its administration to mice with nephrotoxic nephritis ameliorates disease severity and reduces macrophage activation as shown by reduced MHC class II and IL-1β expression and nitric oxide generation [174]. In Thy 1.1 nephritis IL-10 treatment reduced glomerular macrophage infiltration and IL-1β expression, as well as reducing mesangial cell proliferation [175]. By contrast in lupus prone NZB/W F1 mice anti-IL-10 antibodies reduced glomerular inflammation and improved survival, an effect due in part to up-regulation of TNF-α [176]. Which cells are critical for this TNF-α production is unclear. IL-11 inhibits LPS induced TNF-α, IL-1β, and IL-12 in macrophages [177] and administration to WKY rats with nephrotoxic nephritis reduced macrophage infiltration, proliferation and level of proteinuria [178].

One of the functions of macrophages/dendritic cells is to present antigen to T cells. This requires a cognate interaction between MHC class II and the T-cell receptor along with CD80 and CD86 binding to T-cell CD28 providing costimulation. This process can be blocked by soluble CTLA-4 and administration of this to WKY rats with nephrotoxic nephritis reduced antiglomerular basement membrane antibody production, macrophage and T cell glomerular infiltration, and proteinuria [179].

Inhibition of signaling pathways

Most of the approaches outlined above alter macrophage function by activating or inhibiting intracellular signaling pathways and a number of strategies exist to directly affect these. NF-κB is a central regulator of proinflammatory gene expression and inhibition of it by steroids at least in part explains their anti-inflammatory effect in experimental renal inflammation [180]. A more specific approach involves blocking NF-κB by transducing glomerular cells with NF-κB decoy deoxyoligonucleotides (ODN) administered by direct renal artery injection [181]. In rats with nephrotoxic nephritis decoy NF-κB ODN reduced leukocyte infiltration, proteinuria, and proinflammatory cytokine expression. Administration of parthenolide, a plant extract which inhibits NF-κB activation, in Thy 1.1 nephritis, reduced proteinuria

and macrophage infiltration and similarly in antimesangial cell nephritis in mice gliotoxin, a fungal metabolite with similar NF-κB inhibitory activity, reduced proteinuria and hematuria [182]. The MAPK pathway also plays a role in proinflammatory activation of cells and pharmacologic inhibition of p38-MAPK reduced the early development of glomerular inflammation in rat nephrotoxic nephritis [183]. With all of these studies the inhibitors operate on all the cells within the kidney (endothelium, tubular cells, podocytes, and inflammatory cells) as well as cells involved in generation of the immune response out with the kidney, so determining which are the critical sites of regulation will require a more specific approach.

Genetic manipulation of macrophage function

This review has focused on the concept that the pathogenetic or reparative roles of macrophages in inflammatory renal disease are determined by their state of activation and that strategies aimed at altering macrophage activation should have therapeutic benefit. One test of this hypothesis is to directly alter macrophage function ex vivo and determine their effects when these cells are delivered to inflamed kidneys. Genetic modification of macrophages provides such a direct approach to harness the ability of macrophages to regulate inflammation. A number of groups have shown the potential for this in both glomerular and interstitial disease [166, 184, 185]. The most effective method for transduction of macrophages routinely available is recombinant adenovirus which provides good short-term expression in a large proportion of cells, although lentiviruses are more effective for long-term stable expression [186]. The majority of approaches so far have focused on macrophages expressing anti-inflammatory or regulatory cytokines. Systemic injection of macrophages transduced with recombinant adenovirus to express IL-1ra reduced the severity of glomerular inflammation in mice with nephrotoxic nephritis and reduced interstitial macrophage infiltration in a model of unilateral ureteric obstruction [187, 188], with transduced cells producing the cytokine for up to 4 weeks in vivo. We have shown that NR8383 cells expressing rat IL-4 localized efficiently to inflamed glomeruli of rats with nephrotoxic nephritis following direct injection into the left renal artery, produced the cytokine locally and reduced macrophage infiltration, histologic markers of glomerular inflammation, and proteinuria for up to 7 days [167]. In the same model injection of primary cultures of rat BMDM transduced to express IL-10 similarly produced a marked reduction in albuminuria and macrophage activation as demonstrated by reduced MHC class II and ED-3 expression [189]. The impact of IL-10 expressing cells was more pronounced than IL-4 with the most significant differences in injury seen 7 days after a single injection of cells. In these experiments there is no evidence of systemic production of cytokine; however, the localized glomerular production will not only alter the function of transduced macrophages but also affect other infiltrating macrophages, endothelial cells, and resident glomerular cells, including mesangial cells and podocytes.

These latter experiments produced the interesting observation that following injection of macrophages into a single kidney the changes in macrophage activation and histologic markers of inflammation were reduced in the injected kidney and in the contralateral glomeruli. This contralateral effect occurred in the absence of systemic IL-4 or IL-10 expression, with very few transduced cells localizing to the contralateral kidney and could not be mimicked by systemic injection of cells. A similar phenomenon was found in experimental arthritis where transduction of one joint with IL-1ra or TNF-α antagonist reduced inflammation at distant joints [190]. The favored mechanism behind this effect is that the expression of regulatory cytokines within an inflamed site alters the function of dendritic cells which migrate to regional lymph nodes and modifies the systemic immune response. Certainly in mouse arthritis models transducing dendritic cells to express IL-4 suppresses inflammation on systemic administration [191, 192]. These experiments demonstrate that the infiltration of a small number of macrophages, with altered function, is able to produce sustained reorientation of the inflammatory response.

The main problem with the use of replication defective adenovirus to transduce macrophages is that expression is not regulated and is short term. To achieve inflammatory specific expression macrophages can be transduced with two recombinant adenoviruses [193]. The first contains the IL-1β promoter which controls expression of Cre recombinase that in turn excises DNA from a second adenovirus carrying a reporter gene under the control of strong viral promoter, leading to reporter gene expression. Using this approach relevant transgene expression was found in inflamed glomeruli of mice with nephrotoxic nephritis but not in noninflamed sites [193]. To achieve longer term expression in macrophages requires integration of the relevant cDNA into the genome. This can be achieved by retroviral transformation of bone marrow stem cells which then differentiate into macrophages. Bone marrow cells derived in this fashion have been transduced to express IL-1ra. Long-term expression of IL-1ra was found in vivo and development of nephrotoxic nephritis was retarded in mice receiving these cells [194]. For humans such CD34+ stem cells can potentially be obtained from human cord blood. In a recent study human CD34+ cells were retrovirally transduced to express β-glucuronidase as a marker gene and injected into NOD/SCID mice. When these mice were treated with LPS CD14+ cells were recruited to the glomeruli and

secreted the transgene, thus showing that modified human stem cells can mature to macrophages in vivo [195].

CONCLUSION

A wide range of experimental intervention studies have shown that altering the local or systemic inflammatory environment will alter macrophage function. Perhaps more tellingly they have also made other critical observations: (*1*) reduction in injury is associated with changes in markers of macrophage function, (*2*) there is a dissociation between macrophage numbers in inflamed sites and severity of injury when disease is modified, and (*3*) macrophage function can be altered by genetic manipulation to regulate inflammation. Thus macrophage function appears more important then the absolute number of infiltrating cells in the control renal inflammation. As understanding of the heterogeneity of macrophage function in inflammation has increased so has the potential to utilize macrophages to regulate and switch off injury. The future will allow further assessment of macrophage inhibitory receptors and anti-inflammatory mediators to alter renal inflammatory disease. There is also increasing appreciation that "nonimmune" diseases such as diabetic nephropathy and progressive renal failure have a significant inflammatory component which may be amenable to therapy. Finally, the potential for both novel therapeutic agents and genetic modification to bias rather than switch off macrophage function will offer new approaches to treat renal disease.

Reprint requests to David C. Kluth, MRC Centre for Inflammation Research, University of Edinburgh, Teviot Place, Edinburgh, EH8 9AG, United Kingdom.
E-mail: david.kluth@ed.ac.uk

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